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The phylogeny of the genus Brachiomonas

TRACY E. HAZEN

(WITH PLATES 3 AND 4 AND FIVE TEXT FIGURES)

Brachiomonas, one of the oddest and most interesting genera of the *Chlamydomonas* group, has not until now been reported outside of western Europe. The genus was established by Bohlin (**1**) in 1898 with two species, differing in details of form, found in Sweden near Stockholm. The more slender species, *B. gracilis*, has not been reported elsewhere. The type species, *B. submarina*, had been collected about three years earlier by Lagerheim (**6**, p. 7) near Tromsø, Norway, and recorded as a *nomen nudum* which was taken up by Bohlin. A somewhat general distribution of this species on the Norwegian coast has been indicated by Wille (**15**) and Printz (**8**, p. 21). It appears that Dangeard (**3**, p. 74) had observed the same form about ten years earlier than Bohlin, at Luc-sur-Mer, France, but had postponed publishing it until gametes could be found. Chodat (**2**, p. 143, *f. 66*) has recorded the species from Ajaccio, Corsica, showing that it is not exclusively of northern habitat.

West (**12**) in 1908 reported a form collected in brackish marshes at Sheerness, England, near the mouth of the Thames, which he rather hesitatingly referred to *B. submarina*, since it seemed to be somewhat intermediate between Bohlin's two species. In July, 1920, at Cullercoats, near Newcastle-upon-Tyne, and in August at Plymouth, England, I found abundant material in small rock pools, showing forms similar to those described by West, together with specimens agreeing more with Bohlin's type. Earlier in the same season (June 25) I had found on the coast of Norway at Valdersund, near Trondhjem,

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a few specimens which showed precise gareement with Bohlin's description of *B. submarina*. Again a few days later, at Aalesund, south of Trondhjem, I collected typical material, and here in great abundance. At this station some pools were almost exclusively filled with slender forms corresponding to Bohlin's *B. gracilis*; in other pools a mixture of the two species, with perplexing intermediate forms, appeared. Observing pools containing a natural pure culture, one would scarcely question the distinctness of the two species: when both were found together in the same pool, the variety of forms seen presented a very strong suggestion of hybridization between the two species. New material collected in New York in March and April, 1921, has furnished forms (FIGS. 5–7) almost precisely like the slender cells illustrated by West, together with others showing every gradation to the broadest type of *B. submarina*. These slender forms closely resemble Bohlin's *f. 1b* of *B. gracilis*, but I am now convinced that West was entirely justified in including them in the very variable broader species. We seem not to have in America, and I did not find in England the extreme forms represented by Bohlin's *f. 1a* and *1c* of *B. gracilis*. Whether they are sufficiently distinct to warrant the maintenance of the second species is a question which would be more convincingly settled through studies by pure culture methods.

I first found *Brachiomonas submarina* in March, 1907, in a small rock pool on the shore of Long Island Sound at Twin Island, Pelham Bay, New York. It was found in almost pure culture, coloring the water green from its abundance, and persisted in the motile condition for some weeks when brought to the laboratory. Although the same station was visited many times during the following twelve years, no *Brachiomonas* was found until the middle of November, 1919, when it was again abundant in the same pool, and occurred sparingly in neighboring rock hollows of similar character. I found it repeatedly in the same pools until the first of December, in spite of hard frosts and ice formation during these two weeks. The habitat appears to be very like that of Bohlin's type station, small hollows so high above ordinary tide limits as to seem to be filled only with rain water, which are nevertheless sometimes dashed by waves so as to become brackish, as evidenced by taste and by the salt incrustation left on the rock margin upon evaporation. About the time of the collection in 1919, an unusually high tide was reported, and it is possible that this may have washed the

Brachiomonas into the pool from the open sea. Such an occurrence might account for the sporadic appearance of the species at this station, though it is much more probable that it is usually present in a resting state and that conditions were not favorable for the production of zoospores at the time of other visits to the station. A strange point is that, although careful examination of the specimens collected in the spring of 1907 shows them all to be like Bohlin's type, as were also the few specimens found there in September, 1920, and abundant material from March to June and in September, 1921, only a few such individuals were found in the fall of 1919 (FIGS. 1, 2), while most of the material showed a distinct difference in form (FIGS. 8–16). This can hardly be interpreted as a mere seasonal variation. New material collected the last of February of the present season, from pools still coated with ice, consisted very largely of the new obtuse form, but after a week or more in the laboratory this form had mostly been replaced by individuals corresponding to Bohlin's type, present only in small numbers at the time of collection. Whether this is a case of the new type actually giving rise to the standard type through reproduction, or merely an example of the regular succession of growth of different organisms familiar in collections of protozoa, is a matter calling for more extended observation. For the determination of the permanent status of the new form, pure culture methods are highly desirable, but thus far I have been unable to grow *Brachiomonas* on seawater agar, which succeeds admirably with other chlamydomonads of similar habitat. For the present it will be convenient to discuss this divergent plant under the name

BRACHIOMONAS SUBMARINA Bohlin, forma **obtusa** f. nov.

This new form is entirely like Bohlin's type in its genera shape; that is, it possesses a long posterior extension or horn and four shorter median arms directed backwards, often with depressions between them; the anterior portion might be roughly likened to the shape of an umbrella with four ribs. There is often a wedge-shaped beak through which the cilia emerge, though sometimes the apex appears merely rounded. The protoplast may fill the arms and posterior horn entirely (FIG. 8) or partially (FIG. 12) or not at all. The massive chromatophore may be equally variable; that is the protoplasm in the horn and in the arms may be all green, as also in the typical form (FIG. 2), or it may be colorless, sometimes appearing sharply

delimited from the central green mass (FIG. 8), while in other cases the green and colorless regions merge into one another almost imperceptibly (FIG. 1). The chromatophore is here certainly less definitely or constantly organized than in most species of *Chlamydomonas*; in fact it would perhaps be more accurate not to speak of a definite chromatophore but merely of cytoplasm with chlorophyll more or less diffused through it. This variability in the extent of the distribution of the chlorophyll in the protoplasm is very similar to the condition in *Hydrodictyon*, to which Harper (**5**, p. 179) has called attention. West (**12**) states that in his specimens of *B. submarina* the chromatophore never extended into the arms. This was undoubtedly due to the fact that he never saw his material when freshly collected, but only after it had been sent from near the mouth of the Thames to Birmingham. I find that very generally in freshly collected specimens the chromatophore fills the arms and posterior horn, but often retracts from them soon after being subjected to the less favorable light and aeration conditions of captivity.

Imbedded in the chromatophore in a subparietal position, regularly on the side next to the stigma, is a single relatively large pyrenoid; in vigorous young specimens this is generally situated in front of the middle of the cell; often in older individuals it becomes posterior in position. The conspicuous red eye-spot is linear in form; or, sometimes at least, it may be very narrowly wedge-shaped, pointed in front and broader behind; it lies in the depression midway between two arms. Most frequently when the cell is at rest the pyrenoid and stigma lie in a median ventral position (FIG. 6). On the other side of the cell from the pyrenoid the nucleus may sometimes be detected without staining, lying in a colorless mass of cytoplasm, best seen in polar view (FIG. 13). When the pyrenoid lies in its characteristic anterior position the nucleus is likely to be somewhat posterior (FIGS. 15, 17); if the pyrenoid becomes posterior the nucleus is then more often median in position. In older stages vacuoles often develop in the chromatophore (FIG. 4) as described by West (**12**).

In hanging drop cultures, or in jars kept for some time in the laboratory, many individuals may be found which show little or no trace of the four lateral arms (FIGS. 14, 15). Bohlin has shown such a form in one dividing individual. The cell wall is of so firm a character that it is difficult to imagine how the arms

could be modified or suppressed after they are once formed; nevertheless in practically all examples of division seen, I have found the arms well developed before the escape of the daughter cells (FIGS. 20, 21) so that such cells can hardly be looked upon as a reversion to the simpler ancestral form.

The main difference between this new form and Bohlin's type lies in the fact that the arms are here always rounded and obtuse, while in the type form they are sharp pointed and often more slender. The characters of the form may be summarized as follows:

BRACHIOMONAS SUBMARINA Bohlin, forma **obtusa** f. nov. *B.* cellulis vegetativis figura structuraque formae typicae similibus, sed brachiis cornuque posteriore obtusis; cytoplasmate et chromatophoro alias in extrema ipsa extendentibus, alias ex eis plus minusve retractis.

Longit. cellularum 15–32μ (saepius 20–25μ); lat. cell, 15–22μ (saepius ca. 18μ).

Hab. in aquis subsalsis lacunarum saxearum. Twin Island, Pelham Bay, New York; Nov., 1919, Feb.–March, 1922.

Chodat's figures (**2**, *f. 66*) indicate that the form collected in Corsica may be identical with the one here described, though it is possible that the difference between his sketches and those of Bohlin may be due to the personal equation in drawing.

As indicated above, I have found this obtuse form only at one station. Bohlin's type, as represented by my FIG. 1–3, I have also collected early in September during each of the past two years at Bass Point, Nahant, Massachusetts. It should be looked for on the coast of Maine and New Brunswick as well, at any points where rocky ledges occur.

In older cultures numerous cells were found which showed the entire protoplast contracted into a globose mass (FIG. 16). These showed no further development in this material; but in fresh hanging drop cultures of *B. submarina* followed for a much shorter time in Norway, such cells presently became quiescent and developed a new spherical wall closely investing the protoplast. Soon these aplanospores showed their chromatophore turning from green to a tawny tint, and finally the original wall of the zoospores gradually disappeared.

Practically all genera allied to *Chlamydomonas* possess two contractile vacuoles pulsating more or less in alternation, situated near the base of the cilia. In *Brachiomonas* these appear to be entirely absent. One of West's figures (**12**, *pl. 20, f. 11*; **14**, *f. 101 c*), it is true, suggested such vacuoles, but his

beautiful original sketches in color, preserved with his collection of drawings in the South Kensington Museum, show that these spots are brown colored granules suspended in the fluid of a large permanent vacuole. The absence of contractile vacuoles I have been inclined to regard as another mark of the highly specialized character of the genus, since they are regularly to be expected in primitive members of this group and its ancestors. It is possible, however, that this peculiarity is merely an incident due to the special habitat; for Bütschli has remarked the general absence of contractile vacuoles in marine Ciliates and Rhizopods, and D'Arcy Thompson (**11**, pp. 165 and 264) regards this absence in such cases as "no more than a physical consequence of the different conditions of existence in fresh water and in salt." The marine species, *Chlamydomonas brachyura* West, besides exhibiting other points of resemblance to *Brachiomonas*, appears also to have no contractile vacuoles; this species was discovered in association with a new Carteriaceous genus *Platymonas*, in which West (**13**) found two small contractile vacuoles, which, however, "could only be observed with difficulty." In two brackish water species of *Platymonas* which I have investigated with considerable care, I am unable to satisfy myself that I ever see contractile vacuoles. Lewis has just published figures of one of these species, *P. subcordiformis* (Wille) Hazen,* which likewise show no contractile vacuoles. Furthermore, two new brackish water forms of the primitive family Polyblepharidaceae, one a minute species of *Pyramimonas* and the other a new genus most closely related to *Polyblepharides*, which have turned up in my latest collections of *Brachiomonas*, made while this paper was in the hands of the printer, very certainly possess no contractile vacuoles. Over against these several records of their absence, however, attention may be called to their presence (though here very small) in *Chlamydomonas caudata* Wille, discussed below, which must be considered as regularly a marine organism.

Asexual reproduction is accomplished by internal division into four or eight (very rarely perhaps two) daughter cells. The division very generally begins while the cell is swimming actively, and often the typical cell shape is acquired by the daughter cells or zoospores and they move with their own cilia inside the mother cell-wall while it continues in active movement

* Notes from the Woods Hole Laboratory,—1921. *Platymonas subcordiformis* (Wille) Hazen. Rhodora **23**: 249–251. *pl. 133, f. 1–19.* Mr 1922.

by its own cilia. Bohlin states that the first division is longitudinal, and certainly this is regularly the case. Chodat presents a figure (**2**, *f. 66*), and I have seen two or three such cases, where the first division appears to be transverse, but it is quite possible that such an appearance is due to an unusual shifting of the position of the cell contents after a proper longitudinal division. The subsequent divisions have not been described or figured by previous observers, and are ordinarily difficult to follow because of the continual active movement of the mother cell. I was so fortunate as to find one very favorable case in *B. submarina* f. *obtusa*, and later many similar ones in the type form, which I believe to be entirely representative. This individual became quiescent after the first division was completed (FIGS. 22, 23); it presented its anterior pole upward, so that it was possible to see easily that the second plane of cleavage is also longitudinal and perpendicular to the first. Immediately after the second cleavage a slight stretching and shifting of the daughter portions took place, so that one of them appeared somewhat underneath two others (FIG. 24). The third cleavage appears to cut each of the four daughter portions across its longer diameter, that is the plane seems to be essentially transverse to the previous divisions and to the axis of the mother cell. The pyrenoid disappears before the first cleavage occurs, and the stigma fades out during this process. A new and rather small pyrenoid appears in each daughter portion after the second cleavage, and probably another pyrenoid arises *de novo* in four of the eight daughter cells. About an hour after the completion of the third cleavage the eight daughter cells had acquired the typical form of the arms, and also the eye-spot and cilia (FIG. 27); after about another hour they escaped from the mother cell-wall. In certain cases it is clear that the cilia of the mother cell remain connected by a strand of protoplasm to one of the daughter portions during division (FIG. 19) or even until the eight zoospores acquire their final form. Whether this is regularly the case appears doubtful. In material identified as *Brachiomonas gracilis* Bohlin, at Aalesund, Norway, I observed numerous cases of division which followed the course outlined above.

In the case of the production of only four daughter cells it is clear that the cytoplasmic polarity of the mother cell would be directly transferred to each of the new zoospores. But when division proceeds further it is less clear how the

polarity may be transmitted: for, as stated above, the third plane appears to cut the four daughter portions transversely in such a manner that half of the resulting eight would retain the original anterior cytoplasm, while the four posterior zoospores would seem to be under the necessity of regenerating (so to speak) their anterior pole. It is possible that in the slight shifting of the daughter portions following so quickly upon the first two divisions there is actually more rotation than is apparent so that the third division may effect a properly qualitative bipartition, but that appears doubtful from the cases observed. The whole problem of polarity in chlamydomonads is one of great interest demanding a degree of care in observation which hardly any species up to the present has received.

Brachiomonas simplex sp. nov.

This species was first discovered at Aalesund, Norway, in the last days of June 1920, and was also collected three weeks later at Cullercoats, near Newcastle-upon-Tyne, and in August at Plymouth, England. It was found in small rock pools of brackish water, sometimes in association with one of the other species of *Brachiomonas*, then again in practically a natural pure culture.

At first sight the organism looked like a *Chlamydomonas*, many individuals (FIGS. 31, 39) showing somewhat the general oval, posteriorly pointed form characteristic of *C. caudata* Wille (FIGS. 46–50), which occurred near by, though never in the same pools. But presently it was noticed that even the cells most nearly approaching *C. caudata* in form were nevertheless more like *Brachiomonas submarina* in internal organization, showing a similar linear stigma and large lateral and anterior pyrenoid; furthermore many individuals show small bumps or protuberances slightly back of the middle region, immediately suggesting the arms of *Brachiomonas*, though never really attaining that character (FIGS. 28, 29). The posterior horn, often rather elongated, shows a characteristic curve not seen in *B. submarina*. Generally the protoplast was conspicuously furnished with vacuoles, but whether this is a constant character is somewhat doubtful, inasmuch as *C. caudata* in similar pools was often similarly vacuolate at this time. The English material, moreover, was much less vacuolate. There is great variability in the development of the lateral protuberances; if formed at all they appear before the escape of the zoospores

from the mother cell, and the form then attained persists through the life of the individual. In newly formed zoospores, usually, but not always, the protoplast and chromatophore extend into the posterior horn so as to fill it completely. In older individuals the protoplast gradually retracts so that a considerable portion of the horn, or finally all of it is empty. The pyrenoid and red eye-spot regularly lie on the same side of the cell, and the nucleus on the opposite side; as in *B. submarina*, this is most clearly shown in cells momentarily resting with one of the poles directed upward (FIG. 32).

As in the other species, asexual reproduction is accomplished by division of the protoplast into four or eight daughter cells which develop the characteristic form while the mother cell still retains its cilia in active movement (FIGS. 35, 36).

In sexual reproduction, gametes may be formed to the number of thirty-two in one mother cell, which retains its motile condition until they are completely developed (FIG. 40) just as in the case of asexual reproduction. The gametes are similar in form to the asexual cells, but much smaller, lacking a cell wall, and furnished with relatively longer cilia: they also seemed to be more apt to show angularity in the middle region than the asexual cells. Usually there was a slight differentiation between the pair, a smaller and more fusiform gamete conjugating with one slightly larger and more angular (FIGS. 41, 42). Meeting by the ciliated anterior ends, the fusion is lateral, resulting in a quadriciliate zygote of ovoid-pyriform shape which swims actively, and may for some time show the two nuclei, two pyrenoids, and two linear eye-spots (FIG. 43); when momentarily at rest the four long cilia trail backwards. Finally the nuclei fuse, the cilia disappear (FIG. 44) the form becomes spherical, and the thick wall of the resting zygospore is developed (FIG. 45). After some days the cell contents assume a reddish orange color similar to that of the aplanospore of this species and of *B. submarina*.

Brachiomonas simplex sp. nov. *B.* cellulis vegetativis majoribus, plerumque oviformibus quidem sed ad posteriorem partem in cornu curvatum productis, brachiis quae ceterae hujus generis species habent aut omnino suppressis aut evolutis tantummodo in 2–4 gibbos exiguuos subangulares vel rotundatos; ad polum autem anteriorem globosis vel papilla conica parvula exornatis: chromatophoro cellulam prope complente aut non longe in cornu posterius se extendente, pyrenoidem lateralem modo in medio modo a fronte habente, saepe vacuola majuscula

plura praebente: puncto rubro (stigmate) lineari prope pyrenoidem sito: nucleo ad latus oppositum cellulae intra partem chromatophori excavatam complexo: ciliis binis ca. ¼ brevioribus quam cellula instructis.

Propagatio fit per cellulae vegetativae impigre natantis divisionem in 4 vel 8 zoosporas, quae tegumenti scissura erumpunt. Praeterea aplanosporae, primo virides, postea paulatim ad colorem fulvum transmutatae, per contractionem protoplasti in massam globosam ad cellulae partem anteriorem, ut in *B. submarina*, efficiuntur.

Generatio fit per copulationem gametarum inter se subaequalium et nudarum, quae cellulis vegetativis forma quidem similes sunt, sed perparvae et ciliis quam corpus ipsum multo longioribus praeditae, quaeque per divisionem cellulae vegetativae protoplasti etiam in 32 individua parturiuntur. Zygosporae globose, tegumento levi vestitae, diametro ca. 12μ.

Longit. cellularum veg. $30–48\mu$, lat. $18–24\mu$. Longit. gametarum $13–15\mu$, lat. $6–8\mu$; longit. ciliorum ca. 18μ.

Hab. in aquis lacunarum saxearum subsalsis. Aalesund, Norway, 28 June, 1920; Cullercoats, Northumberland, England, 19 July, 1920; Plymouth, England, 11 August, 1920.

Because of the fact that unarmed individuals appear in older cultures of *B. submarina*, one might be tempted to inquire whether *B. simplex* is merely a form of that species. Nevertheless in the natural pure cultures seen both at Aalesund and at Plymouth, *B. simplex* maintained its characteristic simplicity of form from the earliest development of the daughter cells to maturity, and even when it was found intermingled with one of the other species it showed a distinctive character, particularly in the curving of the posterior horn, which rendered it always recognizable. Again, the variability of form exhibited by this new species might readily suggest for it a hybrid origin. We know little of hybridization among algae, but a very interesting preliminary report of its occurrence in *Chlamydomonas* has recently been published by Pascher (7). It may be remarked that Pascher's heterozygous cells showed great diversity in internal organization, while here in *B. simplex* only variability of external form is found. From a careful consideration of all the conditions of its habitat and its behavior, I am convinced that *B. simplex* is to be regarded as a distinct species which represents the transition from *Chlamydomonas* to the more characteristic species of *Brachiomonas*.

There is some resemblance between this species and *Chlamydomonas brachyura* West (**13**) a form which developed in a tank of seawater sent from Plymouth to Birmingham in 1915, and for

which I searched vainly at Plymouth in 1920. Nevertheless since *C. brachyura* lacks a red eye-spot and is pointed instead of rounded at the anterior end, we may perhaps find more suggestion of the origin of *Brachiomonas* in the species mentioned at the beginning of this section, namely,

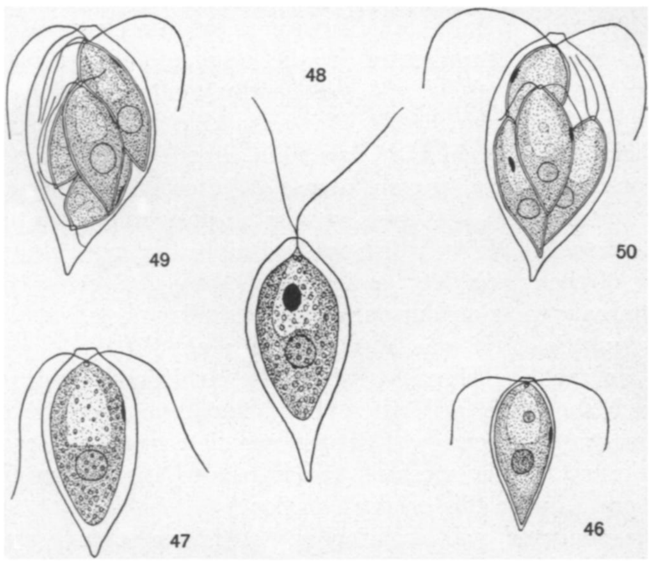
CHLAMYDOMONAS CAUDATA Wille

This very interesting and apparently rare species was found June 28, 1920, at Aalesund, Norway, in small rock pools of the same character as indicated in the original account in 1903 (**15**, pp. 115–118, 135, *pl. 3, f. 4–11*) and my determination of the species was later confirmed by Professor Wille, from living material which I carried to him in Christiania. In one pool was a practically pure culture, giving an intense green color to the brackish water; in another pool close by was a considerable admixture of *C. subcaudata* Wille, which was certainly the most abundant species of the region, now as in 1902. Why *C. caudata* should occur in only three or four out of many similar pools is an interesting question. It was also surprising that it could not be found on the English coast, where the conditions appeared to be much the same, and where I did find several of the other brackish-water species which were associated with *C. caudata* at Aalesund. This species appears not to have been found elsewhere than at this original station.*

The elongated oval posteriorly pointed form of the zoospores is very notable in comparison with the rounded form of the commonly known species of *Chlamydomonas*. The strong resemblance to the more reduced forms of *Brachiomonas simplex* is equally striking; so much so, in fact, as intimated above, that upon superficial observation the two species might be mistaken for closely related members of the same genus. Ordinarily in active individuals, especially in newly formed zoospores, the protoplast conforms rather closely to the shape of the cell-wall, being merely rounded at the point of insertion of

* Playfair, to be sure, has reported [Proc. Linn. Soc. New South Wales **43**: 515. 1918] a form found apparently in fresh water of which he writes: "The only tailed species of *Chlamydomonas*; it is impossible to make any mistake in the identification." Since, however, Playfair states that his specimens lacked pyrenoid and stigma, and his figure shows a distinctly different morphology of the anterior portion of the cell, including a different position of the nucleus, there can be no justification for trying to identify his form with a species so well defined as *C. caudata*, at least until cultural evidence is brought forward to support the attempt.

the cilia, and tapering to an acute point at the posterior end FIG. 46). Older individuals, kept for two weeks or longer in hanging drops, show the cell-wall much thickened, or the protoplast contracted and rounded posteriorly and produced into more or less of a beak at the anterior end (FIGS. 47, 48). The broad truncate anterior papilla of the cell-wall (Hautwarze) impresses me as being rather more prominent than shown in



FIGS. 46–50. CHLAMYDOMONAS CAUDATA Wille

46. Young cell, "dorsal view," showing typica lposition of nucleus, stigma, and pyrenoid. 47. Older cell with thickened wall. 48. Similar cell in "lateral view." 49. Typical arrangement of daughter cells. 50. Unusual arrangement of daughter cells. All, × 720, approximately.

Wille's (15) figures, and the red eye-spot, first described as 'langgestreckter' and again as 'oval oder stabförmig,' was in my mature specimens always a strongly oval disc, sometimes with an anterior pointed end, showing its thinness in the fact that the edge view was narrowly linear. These differences may be readily accounted for by differences of magnification during observation. The longitudinal streaking of the chromatophore noted in the original description, and even introduced in Wille's key to the species, was never distinguishable in vigorous material of either this species or *C. subcaudata* in 1920; only certain individuals kept for ten days or more in

hanging drop cultures showed almost a lattice-like appearance of the chromatophore. Although the chromatophore is hollowed out about as far as the middle of the cell, or sometimes even more deeply, nevertheless the position of the nucleus is notably less central than might be expected, for it lies approximately in the anterior third of the cell.

A point of considerable interest is the position of the stigma and contractile vacuoles. Regularly the eye-spot is so placed that when the cilia are stretched out in a plane parallel to the stage of the microscope the stigma lies on the apparent right side of the cell and in a slightly upper focus, or with the cell revolved 180° the stigma lies on the apparent left side and at a slightly lower focus; in such a position only one of the small contractile vacuoles is visible. If the cell is revolved only 90° from the first position, so that one of the cilia lies nearer the observer and the other at a somewhat lower focus, the stigma shows its disc-like shape in an upper central focus (FIG. 48) and the two contractile vacuoles may be seen side by side at the same time, though one is always smaller than the other, on account of the alternation in their pulsations. This position of the stigma and cilia in one plane and the contractile vacuoles in another plane perpendicular to it indicates a dorsi-ventral character of the cell which appears to me to be the rule in many species of *Chlamydomonas*, though it seems to have received little or no attention from students of the group.*

The dorsiventral differentiation in *Brachiomonas*, as mentioned above and indicated in FIGS. 6, 7, 13, 15, and 32, is slightly different; there the cilia normally stretch out in a plane perpendicular to that which passes through the stigma, pyrenoid, and nucleus, and most commonly the zoospore comes to rest with the stigma lying exactly in the middle on the ventral side, or (less often) on the dorsal side, toward the observer. In many of the figures which seem to indicate a different relative position of the eye-spot and cilia, the latter were actually lying in a plane oblique to that of the microscope stage, which could not easily be shown in the drawings.

* There are, it is true, many published figures of *Chlamydomonas* which show the two contractile vacuoles side by side apparently in the same plane with the cilia; but in cells exhibiting so much movement it is very easy to transfer what is seen in one view to a sketch representing in general a different view, unless one is keenly on the lookout for such a point. Probably also many of our somewhat classic figures are not made from camera lucida drawings of quiescent individuals, as they should be to determine such features.

In the asexual reproduction of *C. caudata*, Wille describes the division as longitudinal, after the disappearance of the cilia of the mother cell, resulting in the formation of four zoospores. On several occasions when I examined material with my travel microscope in the field in late afternoon, or freshly collected material in early evening, I found numerous individuals which contained the four fully formed zoospores while the mother cell was still swimming actively. It was only in the more rarely seen divisions of material kept for a day or two after collection that I found non-motille cells in process of reproduction; such cells may have lost the power of movement earlier because of poorer aeration than in the normal habitat. This division of actively motile cells I have found to be normal in several other species of *Chlamydomonas*. I was unable to find examples which would actually show more of the details of the process of division than indicated by Wille, who merely furnishes one figure of completed division. I did, however, observe that a large majority of cases showed two of the daughter cells with their anterior end directed forwards, and two with the anterior end directed backwards (FIG. 49). In other species of *Chlamydomonas* such as arrangement of the daughter cells is characterstically found when the first plane of division appears transverse to the longitudinal axis of the mother cell, or when, though fundamentally longitudinal, the plane becomes placed transversely by a rotation of the entire protoplast. During the past season I have had the opportunity of observing this latter method many times in a new species to be described in a future paper: this species furnished one exceptional case in which both divisions, carefully followed, were strictly longitudinal, resulting in four zoospores all headed toward the anterior end of the mother cell. This exceptional arrangement I also found in rare instances in *C. caudata* (FIG. 50). Wille's figures (**15**, *pl. 3*, *f. 8*) which shows the same arrangement, appears, therefore to be untypical, and I am obliged to believe that the first division of this species, though properly longitudinal, would regularly show as a transverse cleavage, in consequence of a rotation of the protoplast to such an extent that its anterior pole comes to lie in a position nearly in the middle of the length of the cell.

Reichenow (**9**, **10**, pp. 35–39) it is true, has expressed the view that the division in all species of *Chlamydomonas* may be fundamentally longitudinal, an apparent transverse division being al-

ways preceeded, in his opinion, by a rotation of the protoplast, so that he believes the manner of cell division to have no phylogenetic significance. Attractive as this view is in its possibility of simplifying the idea of direct transmission of polarity to the daughter cells (and my own recent studies on *Chlamydomonas* somewhat tend to support such a view) nevertheless we still lack sufficiently definite and accurate observations to permit the denial that an actual transverse division may occur in some species.

If in *C. caudata* the first cleavage plane is actually or by rotation of the protoplast transverse (as appears to me most probable), and if we accept the current view derived from Dill (4) that the most primitive species are those in which both divisions are longitudinal, then *C. caudata* could hardly be regarded as an actual transitional form between the ordinary ovoid *Chlamydomonas* type and the *Brachiomonas* group, since all species of the latter genus appear to have retained the primitive mode of division by two strictly longitudinal cleavages, in spite of their advancement in other respects. Nevertheless, in its general morphology, and in the similarity of its method of formation of aplanospores (rarely found in other species of *Chlamydomonas*), *C. caudata* must be very close to the ancestral line which has given rise to the four forms of *Brachiomonas* recognized above; and these present a rather close ascending series, with perhaps a definite orthogenetic tendency beginning with *B. simplex*, which is barely emerging from the *Chlamydomonas* stage, through *B. submarina f. obtusa* and then *B. submarina* (type) to *B. gracilis*, an extremely slender form, so far removed from any *Chlamydomonas* type as to justify thoroughly the retention of *Brachiomonas* as a genus.

In certain graphic schemes of the phylogeny of the chlamydomonads—e. g. those of Wille (**15**) and West (**14**, p. 163)—the genus *Lobomonas* Dangeard is indicated as representing an intermediate stage between *Chlamydomonas* and *Brachiomonas*; but the two known species of *Lobomonas*, as well as two new species to be described by the writer in the next number of this journal, are very minute forms, and in their general morphology exhibit by no means so good a connection as is shown by *C. caudata* and *B. simplex*. *Lobomonas* must be regarded as a special offshoot from the *Chlamydomonas* line not leading to anything higher, so far as we know at present.

BARNARD COLLEGE,
COLUMBIA UNIVERSITY

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Description of plates 3 and 4

All drawings made with the aid of camera lucida from living material in hanging drop cultures (or in some cases freshly fixed with osmic acid vapor): PLATE 3 done with Leitz 1/12 in. oil immers. obj. and compens. oc. 6, magnifica-

tion about 1440 diameters; plate 4 done with Spencer 2 mm. oil immers. obj. and Leitz compens. oc. 6, magnification about 1300 diameters; both reduced in reproduction to about 720 diameters.

PLATE 3

FIGS. 1–7. BRACHIOMONAS SUBMARINA Bohlin, type form

FIG. 1. Vegetative cell; posterior horn filled with colorless cytoplasm.

FIG. 2. Chromatophore filling the extremities: Dec. 1919.

FIG. 3. Colorless cytoplasm in extremities; stigma and pyrenoid lying on the under side of the cell: March, 1921.

FIG. 4. Anterior polar view of similar cell; vacuoles in chromatophore.

FIG. 5. Slender form similar to Bohlin's *f. 1b* of *B. gracilis* (fixed with osmic acid vapor).

FIG. 6. Moderately slender form: showing typical position taken by living zoospore temporarily at rest, the pyrenoid and stigma lying in the center of the under side (osmic vapor): April 1921.

FIG. 7. Anterior polar view of similar cell, showing cilia stretching out in plane perpendicular to that in which the nucleus, pyrenoid, and stigma typically lie.

FIGS. 8–27. BRACHIOMONAS SUBMARINA forma OBTUSA Hazen

FIG. 8. Chromatophore contracted, arms and horn filled with colorless cytoplasm (osmic vapor): November, 1919.

FIGS. 9, 10. Arms empty, colorless cytoplasm extending into posterior horn (osmic vapor).

FIG. 11. Anterior polar view of similar cell, living.

FIG. 12. Arms and horn nearly empty (osmic vapor).

FIG. 13. Posterior polar view of similar cell, living, showing typical relative positions of organs as in FIG. 7; a large colorless cytoplasmic region around the nucleus.

FIG. 14. Older cell showing no trace of arms.

FIG. 15. Cell from old culture with only a suggestion of lateral arms; stigma and pyrenoid lying underneath the nucleus.

FIG. 16. Senescent cell with contracted protoplast, preparing to form an aplanospore: December, 1919.

FIG. 17. Young cell showing nucleus posterior to pyrenoid.

FIG. 18. Posterior polar view of similar cell.

FIG. 19. First division of motile cell; 10:30 P. M. (osmic vapor).

FIG. 20. Eight zoospores fully formed in motile mother cell.

FIG. 21. Four zoospores: cilia of mother cell no longer present.

FIGS. 22–27. Successive stages in division resulting in eight zoospores (November, 1919): FIG. 22 at 2:40 P. M.; FIG. 23 at 3:40; FIG. 24 at 4:50; FIG. 25 at 5:30; FIG. 26 at 6:20; FIG. 27 at 7:40. The zoospores escaped before 8:40.

PLATE 4

BRACHIOMONAS SIMPLEX Hazen

Drawn 29 June—4 July, 1920, Aalesund, Norway.

FIGS. 28, 29. Typical mature vegetative cells: in FIG. 29 the stigma lies slightly underneath.

FIG. 30. Anterior polar view of similar cell.

FIG. 31. Cell on which the "bumps" representing lateral arms are undeveloped.

FIG. 32. Anterior polar view of similar cell.

FIG. 33. Protoplast ertracted from one of the "bumps" (osmic vapor).

FIG. 34. Large older cell with more posterior pyrenoid.

FIG. 35 Eight zoospores in motile cell; 12:45 A. M.

FIG. 36. Four zoospores in motile cell.

FIG. 37. Young zoospore free; chromatophore filling posterior horn.

FIG. 38. Three of the four zoospores have escaped through a triangular rent in the wall of the mother cell.

FIG. 39 Similar to FIG. 31, but with more typical position of pyrenoid (osmic vapor)

FIG. 40. Thirty-two gametes in motile mother cell; 10 A M.

FIGS. 41, 42. Gametes at beginning of conjugation (osmic vapor).

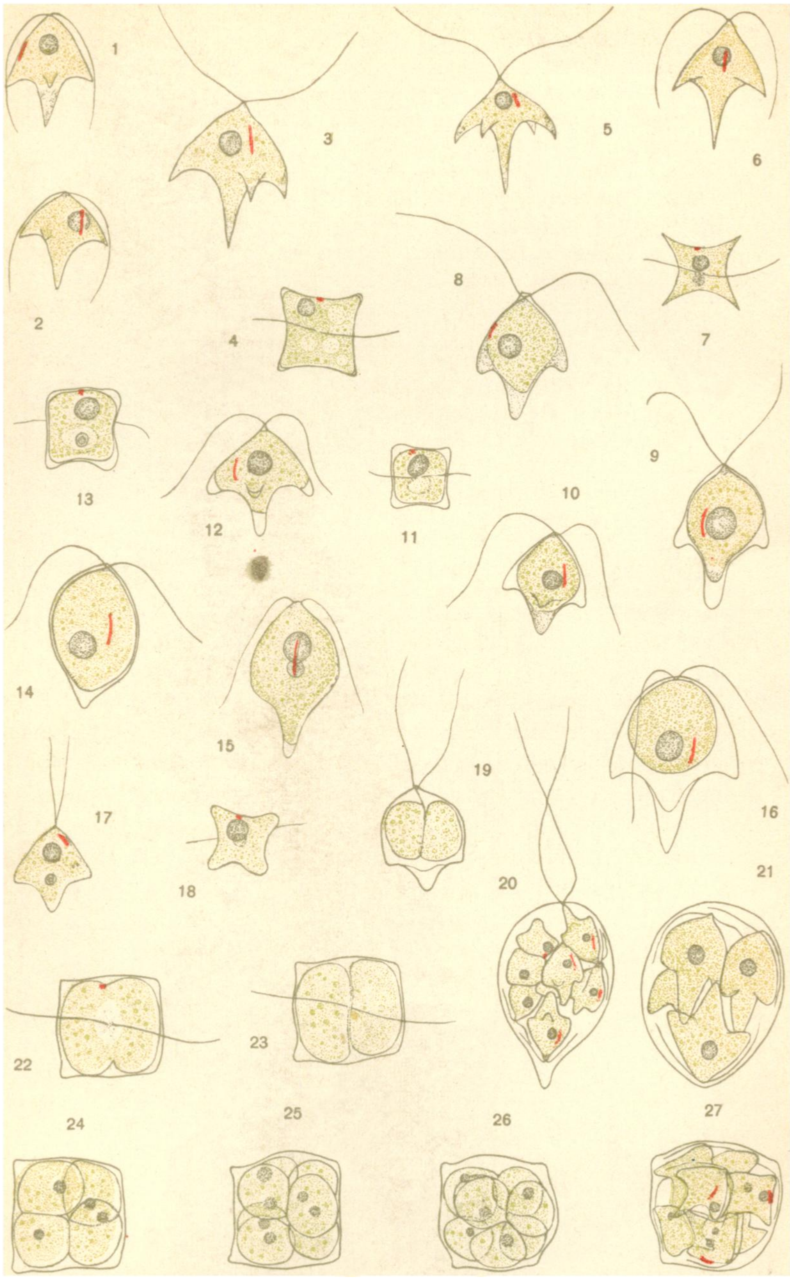
FIG. 43. Motile zygote, momentarily resting; nuclei not fused.

FIG. 44. Zygote, cilia having disappeared, nuclei fused; two stigmata still present.

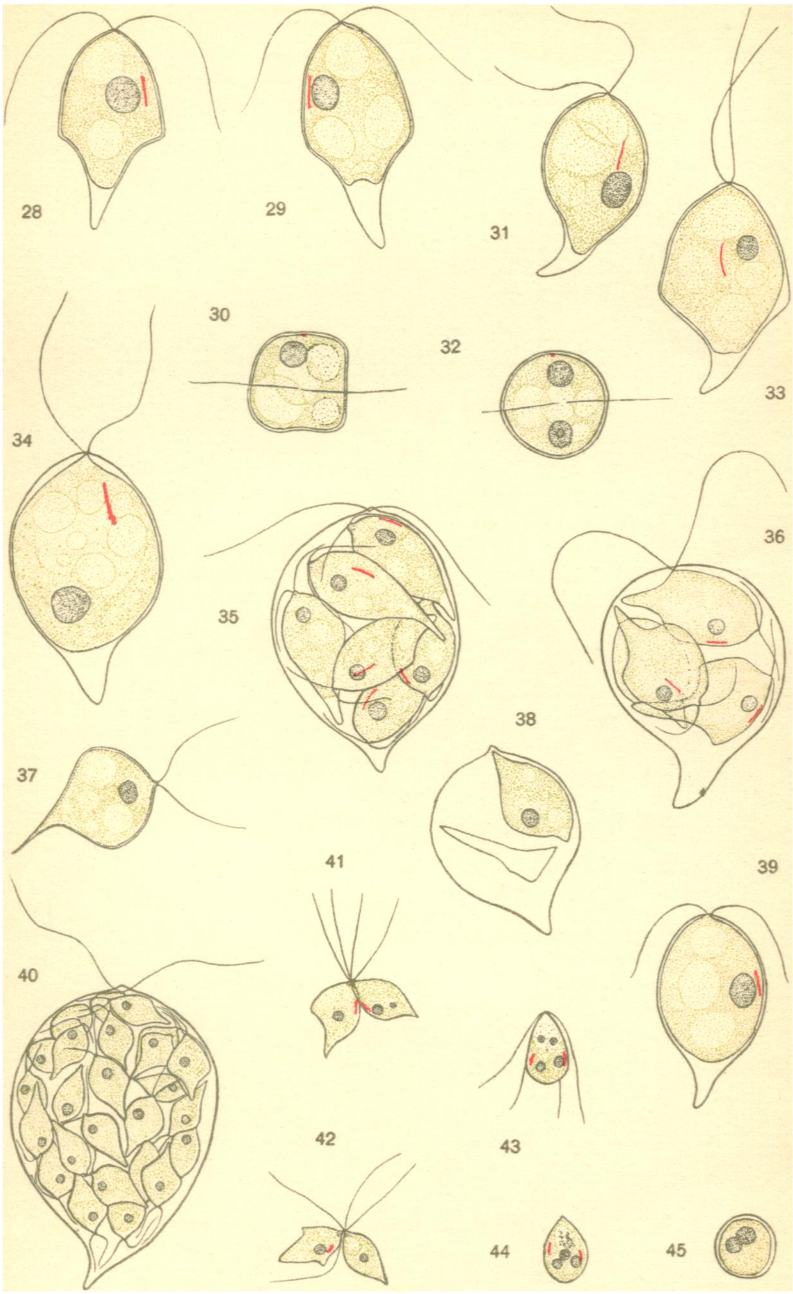
FIG. 45. Zygospore twenty-four hours after conjugation.

In the living cells the pyrenoid actually appears upon casual observation to be green because imbedded in the green chromatophore. It is believed, however, that the printing of the pyrenoids in the same sepia as the cell-wall and protoplasmic outlines represents more truthfully their real nature.

Note. As final proof of this paper is received, the writer has just made a mid-April excursion to the Massachusetts coast, where he has collected typical *Brachiomonas submarina* Bohlin from Cape Ann to Buzzards Bay, at Rockport, Gloucester, Marblehead, Nahant, and Fair Haven. There has been no indication of other species of *Brachiomonas* or of *Chlamydomonas caudata* at these stations.



1-7. BRACHIOMONAS SUBMARINA Bohlin
8-27. BRACHIOMONAS SUBMARINA forma OBTUSA Hazen



BRACHIOMONAS SIMPLEX HAZEN